Nanofludic Devices of Supported Lipid Bilayer Membranes

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Dense 50 nm chromium line patterns on quartz wafer are fabricated by electron-beam lithography with HSQ negative resist. Two dimensional fluidic supported lipid bilayer membranes are deposited over those patterns, which serves as diffusion barriers of membranes. By fluorescence recovery after photobleaching (FRAP) experiments, it shows that the diffusion of lipid bilayer membranes has been confined in one dimension. With these nano-patterned diffusion barriers, multi-components supported lipid bilayer membranes potentially can be achieved, which are very useful tools for studying biomolecular interaction at the cell-cell interfaces , especially immunological/neuronal synapse formation. Future work will focus on the integration of microfluidic channels and hydrodynamic focusing with the e-beam patterns.

I. INTRODUCTION

In a fluid medium , there is continuous diffusive mixing among components. In order to retain spatial information such as a concentration gradient or an image in the absence of external forces, the fluid must be partitioned. For ordinary liquids in three dimensions this can be achieved by constructing containers or with small droplets. However, lipid bilayer membranes , as in figure 1, on solid supports are two-dimensional fluids [1], and are used as a model system to explore the molecular interactions in interfaces. In previous literature, there are many methods for patterning these support membranes, such as scratching[2], stamping lipids with poly-(dimethylsilane) (PDMS) stamps[3], and stamping proteins via microcontact printing.

Moreover, researchers also use micro-patterned grids or Brownian ratchets to partition and sort biomolecules, as in figure 2. Fluorescently labeled lipids are observed to diffuse freely within each membrane corral but are confined by the micropatterned barriers. Application of an electric field parallel to the surface induces steady-state concentration gradients of charged membrane components in the corrals. This system provides an intrinsically parallel means of acquiring information about molecular properties such as the diffusion coefficient in individual corrals.

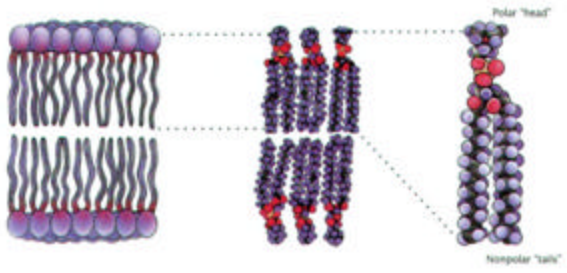

Fig.1. The structure of lipid and lipid bilayer membranes. Polar head is hydrophilic, where as nonpolar carbon tails are hydrophobic.

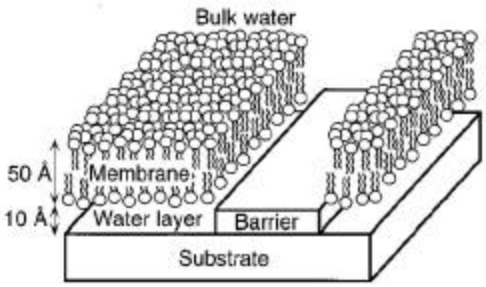

Fig.2. Schematic diagram of a supported bilayer partitioned by a microfabricated barrier. The size of the membrane is exaggerated to illustrate its structure; actual membranes are typically 50 Å thick and are separated from the surface by a ~10 Å layer of water, which provides the intrinsic two dimensional fluidity[5].

By modern electron beam lithography , the optical diffraction limit can be overcome, and line width can be down to 20nm, which is much smaller compared to the cell size. Because synapse formation is contributed by the reorganizations /redistribution of membrane proteins[6] due to the lateral fluidity of cell membranes, nanoscale diffusion barrier with supported lipid bilayer membranes will potentially give great helps in understanding the mechanism of cell-cell signaling. Here we demonstrate the fabrication of dense 50 nm chromium lines as the one dimensional diffusion barriers of fluidic supported membranes.

II. DESIGN

To partition lipid membranes into one-dimensional mobility in large area, a continuous line with snake shape, as in figure 3, is designed with L-Edit Pro (Tanner Research, Pasadena, CA). While the line width is fixed at 50 nm, the spacing between lines varies from 200nm to 2μm. The whole patterns with continuous line wandering inside are ~200μm^2. Because of the high density over

Fig.3. The CAD design of diffusion barriers with 50nm line width. 200nm spacing between lines is shown here. The whole patterns are about 200 μm^2. The drawing is not to scale.

large area, proximity effect of electron must be taken into account during lithography.

III. FABRICATION

50 nm thick Chromium layer is evaporated on 4 inch quartz wafer (Hoya, San Jose, CA) by electron-beam thermal evaporator. Followed by 110° C 5 minute dehydration on the hot plate, 1.8 % hydrogen silsesquioxane (HSQ) negative resist is spun-on at 1000rpm for 45 second. After prebake in 170 °C oven for 5 minute, the wafer is cooled on the chill plate and immediately put into the chamber of e-beam writer.

The patterns are directly written by Leica VB6 100KV thermal field emission nanowriter system with proximity correction and 2000μC/m^2 + 10% dose variation. Then the wafer is immediately developed with LDD-26W (Shipley, Marlborough, MA) for 8 minute, rinsed with DI water and dried by N_2. Under scanning electron microscope (SEM), all features are successfully written.

Chromium strip is performed by Oxford PlasmaLab 100 RIE system (Oxford Instrument, Fremont, CA). The etching rate is ~ 1 nm/min with Cl_2/O_2 in -20 °C. The cross-section of each steps are shown in figure 4.

Finished devices are imaged by scanning electron microscopy (SEM). Most of the features are observed with excellent geometrical shape. However, because of the non-uniform Chromium etching, some lines in the patterns are under-cut, as in figure 5. This non-uniformity etching is solved by adjusting the gases flow rate of RIE system, as SEM images in figure 6.

Fig.4. The cross-section of the pattern on each steps.

IV. EXPERIMENT

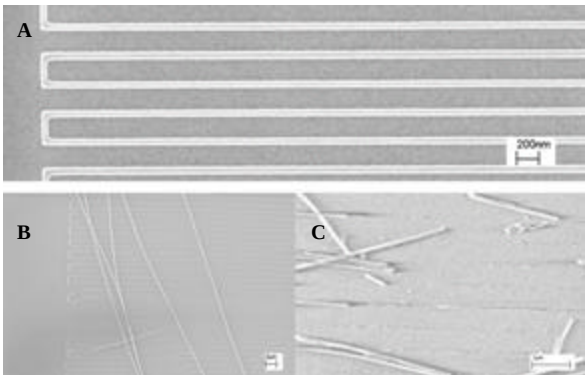

Fig.5. (A) SEM image of patterns before etching Chromium. 200nm line spacing is shown here. (B), (C) After non-uniform chromium etching, some of the patterns are under-cut. Scale bar is 2μm and 1μm, respectively.

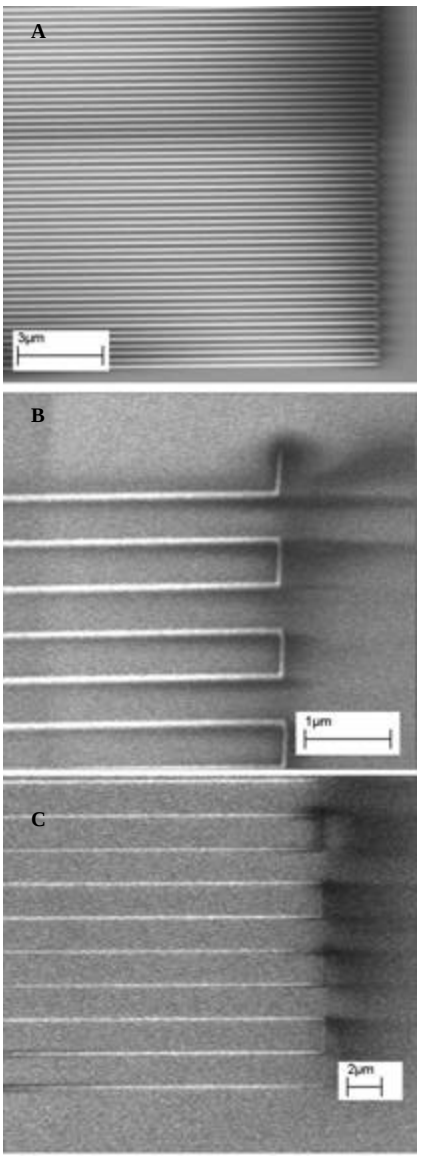

Fig. 6. SEM images of patterns after uniform chromium reactive ion etching. No under-cut, over-etch, or discontinuity observed. Line width: 50 nm. Line spacing: (A) 200nm, (B) 500nm, (C) 2μm

Dimyristoyl-phosphatidylcholine (DMPC), dioleoyl-trimethylammoniumpropane (DOTAP), and 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-l)amino]dodecanoyl]-sn-glycero-3-phospholcholine (NBDPC) are obtained from Avanti Polar Lipids (Alabaster, AL). Texas Red dipalmitoylphosphatidylethanolamine (Texas Red DPPE) is obtained from Molecular Probes (Eugene, OR). All lipids are dissolved in chloroform when purchasing.

Preparation of small unilamellar vesicles (SUVs), has been described previously[7]. In brief, solutions of lipids in chloroform are mixed to the desired composition and the chloroform is then removed by evaporation. The resulting dried lipid films are hydrated with distilled, deionized water (2 mg/mL) at 4°C for approximately 12 h. SUVs are formed either by sonication and ultracentrifugation (166 000g, 2.5 h, 4°C) or by extrusion through 0.1 μm polycarbonate filters.

A 1:1 mixture of SUV solution and phosphate-buffered saline (PBS) is spread over the surface of quartz wafers, which are cleaned previously with UV-Ozone reactor for 10 minute in order to get hydrophilic surface. Then supported membranes form rapidly; excess SUVs are rinsed from the sample. The bilayers are always immersed in an aqueous environment.

Supported lipid bilayer membranes are viewed with a Nikon TE300 inverted fluorescence microscope (Nikon, Tokyo, Japan) equipped with a mercury arc lamp. Images are recorded with a cooled charge-coupled device camera (Hamamatsu C4742-98 ORCAII, Hamamatsu, Japan) through 60X and 20X objectives. Image data is analyzed with Simple PCI (Compix, Cranberry Township, PA).

The fluidity of membranes is examined by FRAP, as in figure 7. The bleached spot becomes brighter as time passes; it confirms the two dimensional mobility of supported membranes. With the same FRAP method, we photobleach the membranes over nano-fabricated patterns. As we expect, the fluorescence recovery only in one direction - parallel with chromium lines, as in figure 8 and 9. Those 50nm chromium line patterns successfully serve as one-dimensional diffusion barriers of supported lipid bilayer membranes.

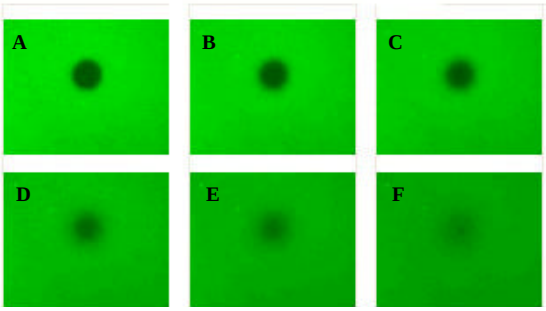

Fig.7. FRAP experiment with membranes on quartz wafer via 60X objective. Membrane composition: 88 mol % DMPC, 10 mol % DOTAP, and 2 mol % NBDPC (green dye). Images (A)-(F) are taken with ~ 3 minute interval. The fluorescence recovery ensures intrinsic two dimensional fluidity of supported lipid bilayer membranes.

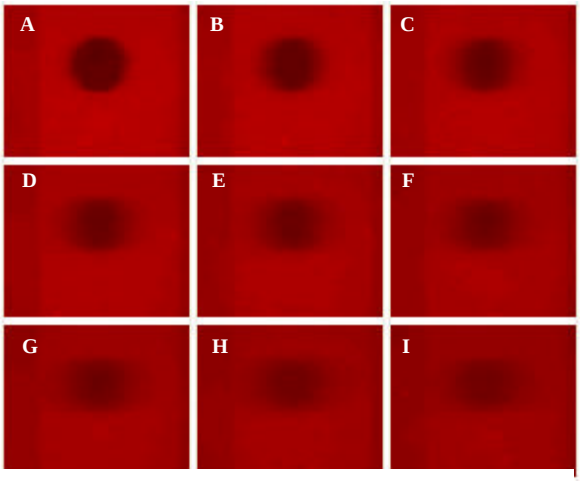

Fig.8. FRAP experiment with membranes over nanofabricated patterns on quartz wafer via 20X objective. Line spacing of this pattern is 2μm. Membrane composition: 88 mol % DMPC, 10 mol % DOTAP, and 2 mol % Texas Red DPPE (red dye). Images (A)-(I) are taken with ~ 3 minute interval. Fluorescence recovery only occurs in one direction - parallel with chromium lines. 50nm chromium line indeed limits the .diffusion against to it.

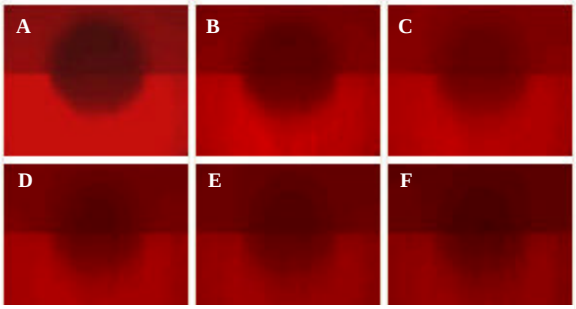

Fig.9. FRAP experiment with membranes over nanofabricated patterns on quartz wafer via 60X objective. Line spacing of this pattern is 1.5μm. Membrane composition: 88 mol % DMPC, 10 mol % DOTAP, and 2 mol % Texas Red DPPE (red dye). Images (A)-(F) are taken with ~ 3 minute interval. While lipid bilayer membranes diffuse freely outside the pattern, the mobility of membranes inside the patterns have been confined by chromium lines.

V. FUTURE APPLICATIONS

By high-resolution electron beam lithography, the width of diffusion barriers can be down to nanometer scale, and high-density patterns of diffusion barriers in large area can also be achieved at the same time. Together with the modern techniques of micro-fluidics and hydrodynamic focusing, as in figure 10, multi-components supported lipid bilayer membranes partitioned by nano-structures potentially can be achieved. This will enable us to study the dynamics of synapse formation.

VI. CONCLUSION

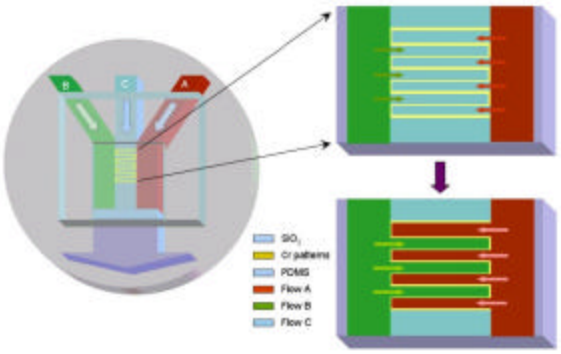

Fig.10. Left: Schematic of fluidic channels with nanoscale patterns. It is based on glass substrate and sealed by PDMS bonding. Right: Intrinsic diffusion property is adapted for filling different bio-molecular into nano-fluidic channels. By the lateral diffusion of supported bilayer membranes, protein A (red) and protein B (green) will automatically recover and be confined in the regions. The diffusion can also be enhanced by applying electronic fields properly.

We successfully fabricated diffusion barriers for supported membranes by electron beam lithography. Dense and continuous 50nm wide chromium lines are achieved, and they indeed limit the direction of membranes diffusion. This encourages us to use nano-patterned substrates and supported lipid bilayer membranes to explore the biological mysteries.

ACKNOWLEDGEMENT

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